

CLAIMS

WHAT IS CLAIMED IS:

- 5 1. An isolated nucleic acid construct comprising a nucleic acid sequence encoding an LTR-type retrotransposon.
2. A nucleic acid construct according to Claim 1 wherein the LTR-type retrotransposon comprises Intracisternal A
10 particle (IAP)-type retrotransposon.
3. A nucleic acid construct according to Claim 1 wherein the retrotransposon comprises a full-length IAP element.
- 15 4. A nucleic acid construct according to Claim 1 wherein the retrotransposon encodes a polypeptide having a function.
5. A nucleic acid construct according to Claim 1 wherein
20 the function comprises at least one activity selected from the group consisting of transcription activity, reverse transcription activity and integrase activity.
6. A nucleic acid construct according to Claim 1 wherein
25 the retrotransposon is an IAP element and at least one domain selected from the group consisting of LTR, *gag*, *pol* and tRNA binding site, which is conserved against SEQ ID NO: 1.
7. A nucleic acid construct according to Claim 1 wherein
30 the retrotransposon is an IAP element, wherein the nucleic acid thereof has at least one feature selected from the group consisting of repeat of a sequence of TCCGGGACGAGAAAA in the tRNA binding site immediately located at LTR at the 5'

side, and inclusion of two or more repeat sequences TTGCTTCTTGCTCTC in the R region.

8. A nucleic acid construct according to Claim 1 wherein
5 the retrotransposon comprises:

(a) a polynucleotide having a base sequence set forth in SEQ ID NO: 1 or a fragment sequence thereof;

(b) a polynucleotide encoding a polypeptide consisting of an amino acid sequence set forth in SEQ ID
10 NO: 2, or 3 and 4, or a fragment thereof;

(c) a polynucleotide encoding a variant polypeptide consisting of an amino acid sequence set forth in SEQ ID NO: 2, or 3 and 4 with at least one mutation selected from consisting of at least one amino acid substitution, addition
15 and deletion, or a fragment thereof, which possesses a biological activity;

(d) a polynucleotide being a splice variant or allelic variant of the base sequence set forth in SEQ ID NO: 1, or a fragment thereof;

(e) a polynucleotide encoding a species homolog of a polypeptide consisting of an amino acid sequence set forth in SEQ ID NO: 2, or 3 and 4, or a fragment thereof;

(f) a polynucleotide which hybridizes to any of polynucleotides (a) through (e) or the complement thereof
25 under stringent conditions, and encoding a polypeptide having a biological activity; or

(g) a polynucleotide having at least 70 % identity to any of polynucleotides (a) through (e) or the complement thereof under stringent conditions, and encoding a
30 polypeptide having a biological activity.

9. A nucleic acid construct according to Claim 1 wherein the nucleic acid sequence encoding the retrotransposon

comprises SEQ ID NO: 1.

10. A nucleic acid construct according to Claim 1 further comprising a promoter sequence.

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11. A nucleic acid construct according to Claim 10 wherein the promoter sequence has an activity of 0.1 rlu or greater when determined by a luciferase assay *in vitro*.

10 12. A nucleic acid construct according to Claim 10 wherein the promoter sequence is selected from the group consisting of CMV, CA and the variants thereof.

13. A nucleic acid construct according to Claim 10 wherein
15 the promoter sequence partially substitutes a portion of 5'LTR of the LTR-type retrotransposon.

14. A nucleic acid construct according to Claim 13 wherein the promoter sequence substitutes an entirety or portion
20 of U3 region in the 5'^LTR in the LTR-type retrotransposon.

15. A nucleic acid construct according to Claim 10 wherein the promoter sequence is operably linked to the retrotransposon.

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16. A nucleic acid construct according to Claim 10 wherein the promoter sequence is located in frame to a transcription initiation site of the retrotransposon at the transcription initiation site of the promoter sequence.

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17. A nucleic acid construct according to Claim 10 wherein the promoter sequence is a base sequence set forth in any of SEQ ID NO: 5-7, or a portion or variant thereof, and

comprises a nucleic acid sequence having promoter activity.

18. A nucleic acid construct according to Claim 10 wherein
the promoter sequence consists of a nucleic acid sequence
5 set forth in SEQ ID NO: 6 or 7.

19. A nucleic acid construct according to Claim 1 further
comprising a sequence encoding a foreign gene.

10 20. A nucleic acid construct according to Claim 19 wherein
the sequence encoding the foreign gene is placed in said
retrotransposon.

21. A nucleic acid construct according to Claim 19 wherein
15 the foreign gene renders a host a distinguishable property.

22. A nucleic acid construct according to Claim 21 wherein
the distinguishable property is selected from the group
consisting of PCR primer, antibiotic resistance, complement
20 of nutrition, enzymatic activity and fluorescence.

23. A nucleic acid construct according to Claim 19,
wherein the foreign gene is selected from the group
consisting of *neo*, GFP, *hyg*, *puro*, *zeo*, *bsr*, *lacZ*, CFP, YFP,
25 RFP, BFP and hrGFP.

24. A nucleic acid construct according to Claim 19,
wherein the foreign gene is composed such that the foreign
gene is first expressed only after transcription, reverse
30 transcription and insertion into the genome it is subjected
to.

25. A nucleic acid construct according to Claim 19, wherein the foreign gene comprises an intron sequence.

26. A nucleic acid construct according to Claim 25,
5 wherein the intron sequence is located in the same transcription direction (forward) with respect to the retrotransposon.

27. A nucleic acid construct according to Claim 25,
10 wherein the intron sequence is located between a splice donor sequence and a splice acceptor sequence.

28. A nucleic acid construct according to Claim 1 for use in genomic modification.

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29. A nucleic acid construct according to Claim 11 which is for confirming whether or not the retrotransposon has transposition ability.

20 30. A nucleic acid construct according to Claim 19 which is for transposing the foreign gene.

31. A nucleic acid construct according to Claim 19 which is used for introducing the foreign gene into a host.

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32. A method for modifying a genome in a cell, comprising the steps of:

A) providing a nucleic acid construct comprising an LTR-type retrotransposon;

30 B) introducing the nucleic acid construct into the cell;

C) culturing the cell for a predetermined period of time; and

D) selecting a cell with a genome modified by means of the nucleic acid construct.

33. A method according to Claim 32, further comprising a
5 promoter having an activity of 0.1 rlu or greater as
determined by a luciferase assay *in vitro*, wherein the
predetermined period of time is sufficient for
transcription, reverse transcription and insertion into the
genome.

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34. A method according to Claim 32, wherein the promoter
sequence is located in frame to a transcription initiation
site of the retrotransposon at the transcription initiation
site of the promoter sequence.

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35. A method according to Claim 32, wherein the nucleic
acid construct comprises a foreign gene located in an
operable manner in the retrotransposon, and the selection
is achieved by the expression of the foreign gene.

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36. A method according to Claim 32, wherein the foreign
gene is located in the reverse direction with respect to
the transcription direction of the retrotransposon, and
comprises a splice donor sequence and splice acceptor
25 sequence, and an intron sequence located *cis*-direction
sandwiched therebetween, wherein said predetermined period
of time is sufficient for achieving transcription, reverse
transcription and insertion into the genome, and wherein
the selection is achieved by the expression of the foreign
30 gene.

37. A method according to Claim 36, wherein the foreign
gene encodes an agent selected from the group consisting

of a antibiotic resistance gene, nutrient supplement agent, enzyme and fluorophore, and the selection is achieved by the property of the cell expressing the agent.

5 38. A method according to Claim 32, wherein the LTR-type retrotransposon comprises an IAP element.

39. A method according to Claim 32, wherein the LTR-type retrotransposon comprises a full-length IAP element.

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40. A method according to Claim 32, wherein the selection is achieved by confirming the transposed sequence by means of ligation mediated PCR.

15 41. A method according to Claim 32, wherein the introduction comprises a format selected from the group consisting of transfection, transformation and transduction.

20 42. A method according to Claim 32, wherein the introduction is achieved in the presence of at least one substance selected from the group consisting of cationic lipids and polyamine reagents.

25 43. A method according to Claim 32, wherein the cell is of the same species as that of the natural host of the retrotransposon.

30 44. A method according to Claim 32, wherein the cell is of the different species as that of the natural host of the retrotransposon.

45. A method for assaying transposition activity of a retrotransposon, comprising the steps of:

5 A) providing a nucleic acid construct comprising a nucleic acid sequence encoding a retrotransposon to be assayed, and a promoter sequence having activity of at least 0.1 rlu as determined by a luciferase assay *in vitro*;

B) introducing the nucleic acid construct into the cell;

10 C) culturing the cell for a predetermined period of time; and

D) detecting the transposition by means of nucleic acid construct.

15 46. A method according to Claim 45, wherein the detection comprises the step of ligation mediated PCR.

20 47. A method according to Claim 45, wherein the detection comprises the step of comparing a genomic database and the sequence obtained by the ligation mediated PCR.

48. A method for producing the transgenic organism, comprising the steps of:

A) providing a nucleic acid construct comprising a nucleic acid sequence encoding a LTR-type retrotransposon;

25 B) introducing the nucleic acid construct into a germ-line cell of a desired biological organism;

C) selecting a germ-line cell with the genome thereof modified in the germ-line cell; and

30 D) regenerating the germ-line cell with the genome thereof modified into a biological organism.

49. A kit for modifying the genome of a cell, comprising:
A) a nucleic acid construct comprising a nucleic acid sequence encoding a LTR-type retrotransposon;
B) means for introducing the nucleic acid construct
5 into a germ-line cell of a desired biological organism; and
C) means for selecting a germ-line cell with the genome thereof modified in the germ-line cell.

50. A kit according to Claim 49, wherein the means for
10 introducing the nucleic acid construct into the cell comprises a transfection reagent.

51. A kit according to Claim 48, wherein the transfection reagent is selected from the group consisting of cationic
15 macromolecule, cationic lipid, polyamine reagent, polyimine reagent, and calcium phosphate.

52. A kit according to Claim 50, wherein the transfection reagent is selected from the group consisting of cationic
20 lipid and polyamine reagent.

53. A kit according to Claim 49, wherein the means for selection comprises at least one of means for detection corresponding to one selected from the group consisting of
25 a PCR primer, antibiotic resistance, complement of nutrition, enzymatic activity and fluorescence.

54. A kit for assaying transposition activity of a retrotransposon, comprising:

30 A) a nucleic acid construct comprising a nucleic acid sequence encoding a LTR-type retrotransposon, and a promoter having an activity of 0.1 rlu or greater as determined by a luciferase assay *in vitro*;

B) means for introducing the nucleic acid construct into the cell; and

C) means for detecting transposition by the nucleic acid construct.

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55. A kit according to Claim 54, wherein the means for detecting comprises at least one means selected from means for detection of at least one of the group consisting of PCR primer, antibiotic resistance, complement of nutrition,
10 enzymatic activity and fluorescence.

56. A kit for producing a transgenic organism, comprising:

A) a nucleic acid construct comprising a nucleic acid sequence encoding an LTR-type retrotransposon;

15 B) means for introducing the nucleic acid construct into a germ-line cell of a desired organism;

C) means for selecting a germ-line cell with the genome thereof modified in the germ-line cell; and

20 D) means for regenerating the germ-line with the genome thereof modified into an organism.

57. A kit according to Claim 56, wherein the means for regenerating the organism comprises an organism as a host.

25 58. A promoter comprising a cytomegalovirus enhancer and avian beta-actin promoter, wherein at least one of the cytomegalovirus enhancer and the avian beta-actin promoter comprises a sequence shorter than the native full-length thereof.

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59. A promoter according to Claim 58, wherein the shorter sequence is due to the deletion of a sequence downstream of the transcription initiation site.

60. A promoter according to Claim 58, wherein all the sequence down stream of the transcription initiation site is deleted.
- 5 61. A promoter according to Claim 58, wherein a portion of a sequence downstream of the transcription initiation site and the promoter region is deleted.
- 10 62. A promoter according to Claim 58, wherein the cytomegalovirus enhancer comprises a sequence set forth in SEQ ID NO: 36 and a variant thereof.
- 15 63. A promoter according to Claim 58, wherein the avian beta-actin promoter comprises a sequence set forth in SEQ ID NO: 8 or a variant thereof.
- 20 64. A promoter according to Claim 58, comprising the sequence set forth in SEQ ID NO: 6.
- 25 65. A promoter according to Claim 58, comprising the sequence set forth in SEQ ID NO: 7.
66. A promoter according to Claim 58, consisting of the sequence set forth in SEQ ID NO: 6.
- 25 67. A promoter according to Claim 58, consisting of the sequence set forth in SEQ ID NO: 7.
- 30 68. Use of an LTR-type retrotransposon for genomic modification.
69. Use of a promoter having an activity of 0.1 rlu or greater as determined by a luciferase assay *in vitro*, for

modification of a genome.

70. Use of a promoter having an activity of 0.1 rlu or
greater as determined by a luciferase assay *in vitro*, for
5 confirmation of an LTR-type retrotransposon.